PREPARATION OF COMPOSITE NANOFIBRES FOR BONE TISSUE REGENERATION BY ELECTROSPINNING

A Thesis submitted in partial fulfilment of the requirements for the degree of

Bachelor of Technology
in
Biomedical Engineering

By
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CERTIFICATE

This is to certify that the thesis entitled, **"Preparation of composite nanofibres for Bone Tissue Regeneration"** submitted by **Monica Tudu (108BM007)** in partial fulfilment of the requirements for the award of **Bachelor of Technology Degree** in **Biomedical Engineering** at National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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ABSTRACT

Electro spinning technique is used to produce continuous nanofiber for different application area like preparation of scaffold, microbial filtration membrane, gas filtration membrane and controlled drug delivery system. The present study is an attempt to develop composite nanofibers that are biomimetic and bioactive and accordingly promote cell-scaffold interactions while being used as scaffolds for engineering tissues. The approach proposed involves compounding (hybridizing) two different polymers, both of which are biocompatible and biodegradable, but one is synthetic and the other is of natural origin. They are incorporated into one composite nanofiber from random blending or forming into a core-sheath structure via an advanced electrospinning technique. Composites in the form of nanofibers have been obtained by electrospinning technique. A gelatin/PMMA (Poly Methyl-Methacrylate) nanofibrous scaffold was prepared using Electro-spinning Technique to mimic both the physical architecture and chemical composition of natural bone ECM (Extra Cellular Matrix). With the help of electrospinning technique composite nanofibers were fabricated and it was found that with 45:55 gelatin:PMMA concentration was the most suitable composition for spinning to obtained fiber. This 45:55 ratio composite scaffold was then characterised using various techniques like SEM (Scanning Electron Microscopy), FTIR (Fourier Transform Infrared Spectroscopy), EDS (Energy Dispersive Spectroscopy). The gelatin/PMMA nanofibers obtained under optimum composition of the solution are again electrospun by varying the feed rate values to see its affect on the fiber morphology. Thus it was found that with the feed rate 0.3 ml/h, the nanofibers formed had an average diameter in the range of 250-300 nm and had suitable properties mostly desired for bone tissue regeneration

Contents

Chapter 1

INTRODUCTION

Transplantation of tissues and organs is one of the greatest medical accomplishments of all time. Accidents and diseases results in tissue losses and organ failures which is devastating and a life threatening situation. Currently autograft and allograft transplantation are the two major approaches used to repair or replace damaged or lost tissue and organs. Though autografts avoid an immune response in the patient after re-implantation and cause no pathogenic transmission problems, are associated with limitations such as donor site morbidity and limited availability. On the other hand allografts triggers an immune response in the host body, also carry the risk of disease transfer. The lack of sufficient quantity of donor tissues and organ required the development of a new technology called tissue engineering.

Tissue engineering provides long-term solutions, which is much safer than other options (auto/allografts) and is cost-effective. The presence of residual foreign material is eliminated as well. It provides a novel way to recover physiological function by seeding cells onto scaffolds constructed of natural or artificial materials, together with the use of growth factors and other signalling molecules to modulate cell proliferation and differentiation.

Tissue engineering has obstacles or challenges to face related to cell isolation and preparation, biomaterial design, optimization of nutrient transport and transplantation complexity. Active seeding has some technical difficulties and obstacles to grow cells in sufficient quantities, urging their differentiation into the desired cell type, ensuring their blood and nutrient supply after implantation in the body. Three-dimensional porous scaffolds are required to support the formation of new tissue and extra cellular matrix (ECM), for cellular migration and for the transport of nutrients and metabolic wastes. High porosity, large surface area, suitable pore size, highly interconnected pore structure, biocompatibility,

biodegradability, non- toxicity and structural integrity are important parameters that affect tissue in-growth, allow a high density of seeded cells and promotes neo-vascularization when being implanted in vivo. Both natural polymers and synthetic polymers have been extensively investigated as biodegradable polymeric biomaterials. Biodegradable scaffolds, serve as transplant vehicles for cultured cells and templates to guide tissue regeneration, play an important role in transforming the cultured cells to a new tissue. Natural biomaterials are enzymatically degradable and tend to be biocompatible. In our current work gelatine and poly methyl methacrylate composite nanofibers are being prepared by dissolving the both in a common solvent.

SCOPE OF THE THESIS-

The whole thesis is composed of 7 Chapters and is organized as follows.

a) Chapter 1 gives an introduction of the research background, objectives, and scope of this project.

b) Chapter 2 is a literature review on the electrospinning technology, scaffold technology for tissue engineering, a survey of the prior arts of materials hybridization for scaffold fabrications, and the state of the art of electrospun nanofibers as scaffolds for engineering tissues.

c) Chapter 3 is based on materials and methods and the procedure of electrospinning of gelatin nanofibers.

d) Chapter 4 is results obtained by various characterization techniques of composite nanofibers.

e) Chapter 5 is the final conclusion of the results obtained.

f) Chapter 6 is all about Future work and suggestions.

g) Chapter 7 consists of list of references.

Chapter -2

2.LITERATURE REVIEW

2.1 Nanofibers

Nano-fibers are defined as the fibers whose diameter ranges in the nanometer range. This have a special property of high surface area and increased porosity which makes it favourable for cell interaction and hence it makes its a potential platform for tissue engineering. The high surface area to volume ratio of the nanofibers combined with their microporous structure favors cell adhesion, proliferation, migration, and differentiation, all of which are highly desired properties for tissue engineering applications. [1,2]. There are mainly three techniques involved for synthesising nanofibers namely electrospinning, self- assembly, and phase separation .

2.2 Phase Separation -

In this technique water – polymer emulsion is formed which is thermodynamically unstable. At low gelation temperature, nanoscale fibres network is formed, whereas high gelation temperature leads to the formation of platelet-like structure. Uniform nanofiber can be produced as the cooling rate is increased. Polymer concentration has a significant effect on the nanofiber properties, as polymer concentration is increased porosity of fiber decreased and mechanical properties of fiber are increased. The final product obtained is mainly porous in nature but due to controlling the key parameters we can obtain a fibrous structure. The key parameters involved are as follows.

a) Type of polymers and their viscosity
b) Type of solvent & its volatility.
c) Quenching temperature.
d) Gelling type.

2.3 Self Assembly.

It is a powerful approach for fabricating supra molecular architectures. Self-assembly of peptides and proteins is a promising route to the fabrication of a variety of molecular materials including nanoscale fibers and fiber network scaffolds.[3,4,5,6,7,8]. The main mechanism for a generic self-assembly is the intermolecular forces that bring the smaller unit together.

2.4 Electrospinning

It is a term used to describe a class of fibers forming processes for which electrostatic forces aare employed to control the production of the fiber. Electrospinning readily leads to the formation of continuous fibers ranging from 0.01 to 10 µm. Electrospinning is a fiber forming processes by which electrostatic forces are employed to control the production of fibers. It is closely related to the more established technology of electrospraying, where the droplets are formed. "Spinning" in this context is a textile term that derives from the early use of spinning wheels to form yarns from natural fiber. In both electrospinning and electrospraying, the role of the electrostatic forces is to supplement or replace the conventional mechanical forces (e.g. hydrostatic, pneumatic) used to form the jet and to reduce the size of the fibers or droplets, hence the term "electrohydrodynamic jetting". If this jet will form continuous fiber then it would be treated as electrospinning process. This electrospinning process may be broken down into several operational components:

(i) charging of the fluid,

(ii) formation of the cone-jet,

(iii) thinning of the jet in the presence of an electric field,

(iv) instability of the jet, and

(v) Collection of the jet (or its solidified fibers) on an appropriate target.

2.4.1 History Of Electrospinning

The first patent for electrospinning setup was issued to Formhals in the year 1934 (US patent 1-975-504). In the past several decades, this technique has been used to create fibers from a wide range of polymers including biopolymers, engineering plastics, conducting polymers, and polymer blends.

In the late 16th century William Gilbert describe the behavior of magnetic and electrostatic phenomena. He observed that when a suitably electrically charged piece of amber was brought near a droplet of water it would form a cone shape and small droplets would be ejected from the tip of the cone: this is the first recorded observation of electro spraying.

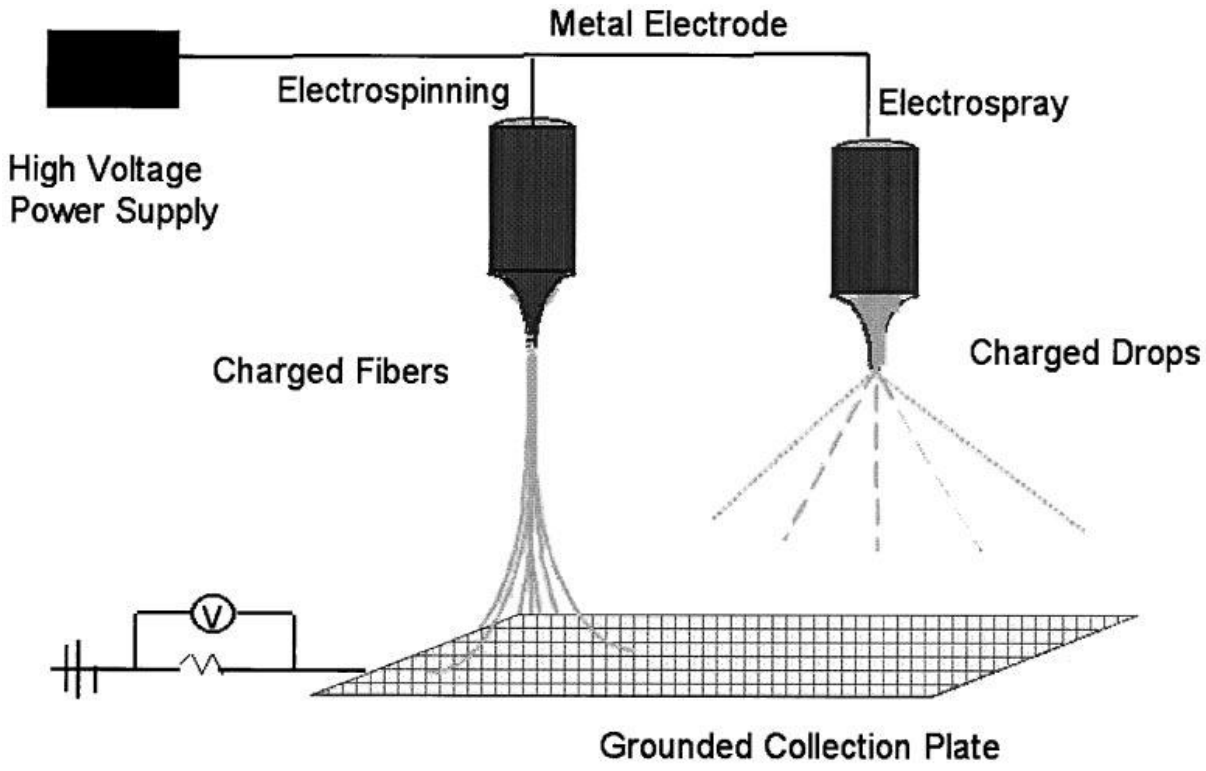
The process of electrospinning was patented by J.F. Cooley in May 1900 and February 1902 (U.S. Patent 692,631) and by W.J. Morton in July 1902 (U.S. Patent 0,705,691 .

Between 1964 and 1969 Sir G. I. Taylor proposed that the fluid droplets make a cone when it ejected from the tip of the syringe under the effect of an electric field; this characterized droplet shape is n known as the Taylor cone.

In the 1990s Reneker and Rutledge proposed that many organic polymers can be electrospun into nanofibers by electrospinnig. After that, the number of publications about electrospinning increased exponentially.

Since 1995 Reznik et al. describes the extensive work on the shape of the Taylor cone and the subsequent ejection of a fluid jet.

2.4.2 Working principle of electrospinning



Electrospinning process involves the following 5 important steps-

2.3.3. Charging of the polymer fluid

2.3.4. Formation of the cone jet (Taylor cone)

2.3.5. Thinning of the jet in the presence of an electric field

2.3.6. Instability of the jet

2.3.7. Collection of the jet

2.4.3. Charging of the polymer fluid

Solution poured in the syringe is filled with an polymer solution, the polymer solution is charged to a very high potential around 10-30kV. The nature of the fluid and polarity of the applied potential free electrons, ions or ion-pairs are generated as the charge carriers forming an electrical double layer. This induction charging is suitable for conducting fluid. But for non-conducting fluid charges may be directly injected into the fluid by the application of electrostatic field.

2.4.4. Formation of the cone jet (Taylor cone)

The polarity of the fluid depends upon the voltage generator .The repulsion between the similar charges at the free electrical double layer work against the surface tension and fluid elasticity in the polymer solution to deform the droplet into a conical shaped structure known as Taylor-cone. Beyond a critical charge density Taylor-cone becomes unstable and a jet of fluid is emitted from the tip of the cone.

2.4.5. Thinning of the jet in the presence of an electric field

The jet seeks a path to the ground, this fluid jet then forms a slender continuous liquid filament. The charged fluid is accelerated in the presence of electrical field. This region of Fluid is generally linear and thin.

2.4.6 Instability of the jet

These Fluid elements are accelerated and thus stretched and succumbs to one or more fluid Instabilities which distort as they grow following many spiral and distort path before collected at the collector electrode. This region of instability is also known as whipping. region.

2.4.7 Collection of the jet

Charged electro spun fibers travel downfield until impact with a lower potential collector plate. Orientation of the collector affects the alignment of the fibers. Different type of collector used are- Rotating drum collector, moving belt collector, and rotating wheel with bevelled edge, multifilament thread, parallel bars, simple mesh collector**.**

2.4.8 Optimization of electrospinning process

Table 1: Different parameters affecting electrospinning process.

a. Solution related parameters	**b) Processing parameters**	**c) Environmental Parameters**
• Molecular weight	• Voltage	• Temperature
• Solution viscosity	• Feed rate	• Humidity
• Surface tension	• Temperature	• Air velocity inside the chamber
• Solution conductivity	• Effect of collector	• Pressure
• Dielectric effect of solvent	• Diameter of the orifice of needle	

A wide range of polymers has been used to electrospin nanofibers. Natural polymers such as collagen, gelatin chitosan, hyaluronic acid and silk fibrion have been used to produce nanofibers that can form potential scaffolds for tissue engineering applications.[3,4,5,6,7]

Among the synthetic polymers explored for the fabrication of nanofibers, poly(lactic acid) (PLA) polyurethane (PU) poly(ε-caprolactone) (PCL) poly(lactic-co-glycolic acid) (PLGA), poly(ethylene-co-vinylacetate) (PEVA) and poly(l-lactide-co-ε-caprolactone) have been well studied.[8,9,10,11]

Natural polymers offer the advantage of being very similar, often identical, to macromolecular substances present in the human body. Therefore, the biological environment is prepared to recognize and interact with natural polymers favorably. Some of the natural polymers used as biomaterials are collagen, hyaluronic acid, gelatin, chitosan [12]. Gelatin has improved mechanical properties and wettability as compared to other natural polymers [13]. In addition, the nanofibrous scaffold of gelatin composite show good cell attachment, growth, and migration of bone marrow stromal cells.

Natural polymers offer the advantage of being very similar, often identical, to macromolecular substances present in the human body. Therefore, the biological environment is prepared to recognize and interact with natural polymers favorably. Some of the natural polymers used as biomaterials are collagen, hyaluronic acid, gelatin, chitosan [12].

Synthetic polymers represent the largest class of bio-materials [13]. A wide variety of synthetic polymers has been used to form nanofibers composite. These include PLA [14,15]; poly(ethylene terephthalate) (PET)[16] for blood vessel tissue engineering; and several copolymeric compounds such as PLLA-CL as a biomimetic ECM for smooth muscle and

endothelial cells [17,18].(PLGA) [19] is one of the most commonly used polymers to fabricate nanofibers for bone and cartilage tissue engineering and controlled drug delivery.

2.4.9 Nanofibrous composite for bone tissue regeneration.

For bone tissue engineering, scaffolds with a pore size in the range of 100–350 μm and porosity greater than 90% are preferred for better cell/tissue in-growth and hence enhanced bone regeneration [20]. Many materials both natural and synthetic have been explored as nanofibrous composite materials for bone, cartilage, ligament, and skeletal muscle tissue engineering, which includes Hydroxy Apatite [21,22], chitosan [23], PLGA [22], carbon [24] and aluminum nanofibers [25].

[26] developed PAN containing gelatin fibers for tissue enginnering purposes. The results obtained suggested that PANi-gelatin blend nanofibers provides a novel conductive material well suited as biocompatible scaffolds for tissue engineering. In another study [27] used gelatin with Poly lactic acid. Their results revealed that addition of gelatin into PLA markedly improved the wettability of the nanofibrous substrate. In [28] it was found that the nanofibrous composites formed had superior property and the bone cells proliferated more.

2.5 Objectives

The overall objective of this project is to develop composite nanofibers that are biomimetic and bioactive and can accordingly promote cell-scaffold interactions while being used as scaffolds for engineering tissues. The approach proposed involves compounding (hybridizing) two different polymers, both of which are biocompatible and biodegradable, but one of synthetic and the other of natural origin. They are incorporated into one composite nanofiber from random blending or forming into core-sheath structure via an advanced electrospinning technique. Biopolymer gelatin (or collagen) and synthetic PMMA are selected as our representative model polymers from the natural and synthetic sources, respectively to perform the following scope of work:

1) To develop a means to electrospin biopolymer of gelatin into nanofibers.

This is to make the generated gelatin nanofibers a practical nanofiber material as useful as its counterpart forms such as films, large-diameter fibers and microspheres, and to provide feasibility for subsequent fabrication of Gt/PMMA composite nanofibers.

2) To fabricate Gt/PMMA composite nanofibers, and characterize their physical and mechanical properties of the resultant composite nanofibrous structure.

Chapter 3

3.MATERIALS AND METHODS

3.1 Materials

a) Gelatin

b) PMMA (poly methyl methacrylate)

c) Glacial acetic acid (GAA)

d) Trichloro acetic acid (TCA)

3.2 Steps Involved for the preparation of the Solvent

1. First of all a solution was prepared using Trichloro Acetic acid and Glacial Acetic acid 10 ml each in a beaker making it upto a final solution of 20 ml.

2. Then PMMA was taken and the required amount in gm was measured in an electronic weighing balance.

3. At normal temperature TCA crystallizes, therefore it is heated in a hot water bath to make it in a solution form.

4. Then it was added to GAA in a beaker to which the measured PMMA crystals were added.

5. This solution was left on a magnetic stirrer for more than 12 hours for PMMA to dissolve uniformly to form a homogenous solution .

6. After that gelatin was measured in the required amount in a measuring weighing balance and was added to the solution after PMMA crystals got dissolved forming a clear solution. It is added 2-3 hours prior electrospinning.

3.3_Electrospinning of gelatin/PMMA nanofibers

To make electrospun fibres, solution of proprietary composition was prepared. Gelatin & PMMA was taken in different ratios (40:60, 45:55, 50:50) and was then dissolved in TCA(Tri-chloroacetic Acid) and GAA(Glacial Acetic Acid) solution. Then this solution was then loaded into a plastic syringe and this was then placed inside the electrospinning machine. A blunt-ended 20-gauge stainless steel needle was used as the nozzle. The emitting electrode from a Gamma High Voltage Research ES30P power supply capable of generating DC voltages up to 30 kV was attached to the needle. The grounding electrode from the same power supply was attached to a piece of aluminium foil which was used as the collector plate and was placed approximately 7 cm below the tip of the needle. Upon the application of a high voltage ranging between 9 and 22.5 kV across the needle and the collective plate, a fluid jet was ejected from the nozzle. As the jet accelerated towards the collector, the solvent evaporated, leaving only ultrathin fibers on the collector. The obtained fibers were left exposed to moisture for approximately 5 h to allow complete hydrolysis. Following parameters involved in formulation was studied

- *Determination of electrospinnability of the fibers.*
- *Determination of morphology and size of fibers obtained using scanning electron microscope (SEM*)
- *Determination of interaction between gelatine & PMMA using FTIR.*
- *Determination of porosity of the fibers.*

Gelatin and PMMA solution were prepared in different ratios taking TCA and GAA as a solvent.

Table 2: Different composition of solution for electrospinning

Sl.Nos.	Ratio	Quantity (in gms)		Temperature (in ^{0}C)	Humidity (In %)
		Gelatin	PMMA		
1.	40:60	2.00	3.00	19	69
2.	45:55	2.25	2.75	27	45
3.	50:50	2.50	2.50	35	23

EDGE type collector is used for the production of nanofibrous composite.

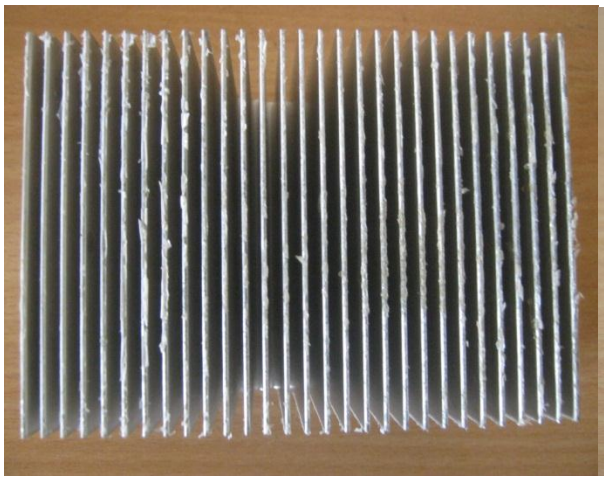


Fig.2 Top view

Fig.3 lateral view

3.4 Fiber characterization

3.4.1 Morphological Analysis

The fiber morphology and the texture, dimensions of the fiber was studied using scanning electron microscope with an accelerating voltage of 20 kv and the obtained image was analysed using Image Tool software for the calculation of the average diameter of the nanofiber. A scanning electron microscopy (SEM) is used to check the surface morphology of any sample. It is a type of electron microscope that takes images of the sample by scanning it with a beam of electrons in a faster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity. In this study, SEM was used for studying fibre morphology and the surface texture of the electrospun nanofibers. In this a small piece of the composite was taken and was placed on the SEM multi-holder. Then it was inserted inside into the chamber and after a while with the application of high accelerating voltage the surface morphology was finely focussed and was studied.

3.4.2 Analysis of chemical composition

EDS has characterization capabilities because each element has a unique atomic structure allowing unique set of peaks on its X-ray spectrum. In this EDS, high energy particles are incident on to the surface of the sample. EDS tells about the chemical composition of the coating layer of the coated surface.

Investigation of the chemical composition of the nano fiber was done using enegy dispersive x-ray spectroscopy for which the amount of different element present was calculated. This was done simultaneously with the SEM.

3.4.3Analysis of chemical interaction.

It was carried out with the help of FTIR spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through or transmitted. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. It identifies chemical bonds in a molecule by producing an infrared absorption spectrum. It is an effective analytical tool for detecting functional groups and characterization covalent bonding information. The bonds such as N-H, C-O ,C=O ,C-O-C, typical FTIR spectrum was found. In this technique firstly scaffold was taken and it was mixed with small amount of KBr. Then it was powdered and then a pellet was formed which was then inserted into the chamber. Then the sample of interest was taken and the light is directed onto it and the intensity is measured using an infrared detector.

Chapter 4

4.1 Results And Discussion

In the present section the results obtained under different processing conditions are discussed in details. Table 1 shows the details processing parameters and solution composition of the experiments under taken.

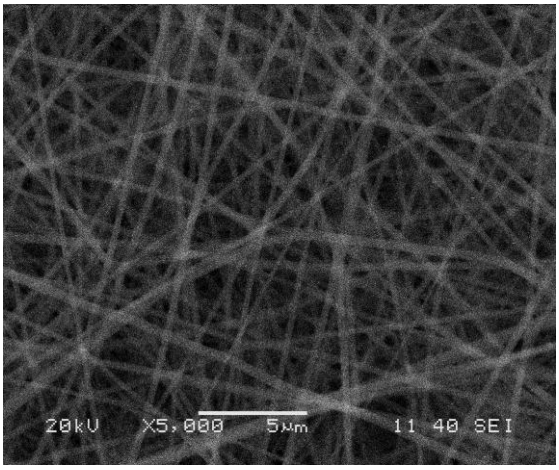
Table.1 The detailed process parameters of electrospinning of the composite nano fiber

Gelatin/PMMA Conc	**Voltage applied (In kV)**	**Time taken (in hr)**	**Possibility of fiber formation**
40:60	20-22	10-12	No
45:55	20-22	10-12	Yes
50:50	20-22	10-12	No

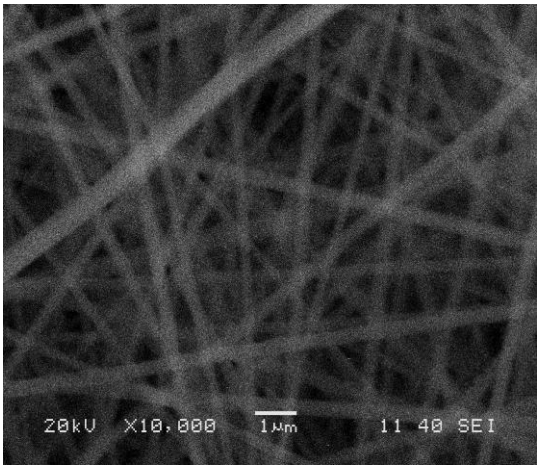
4.2 Effect of solution composition in the formation of fibers.

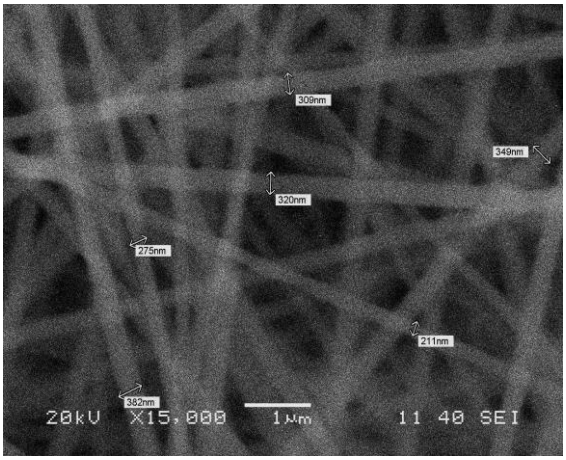
The present section comprises of the gelatin/PMMA nanofiber under different processing parameters were obtained. Nanofibers obtained from the composition 45:55 were examined by scanning electron microscopy. For the concentration 50:50 and 40:60 scanning electron microscopy images were not possible to take as the compositional parameters were not suitable for carrying the electrospinnig process as a result fibers were not formed .

a) For magnification value of 5000



b) For magnification value of 10000





c) For magnification value of 150000

Fig 4.2.1. **Scanning Electron Microsgraph of gelatin:PMMA in 45:55 composition for under a) Magnification of 5000, b) Magnification of 10000. c) Magnification of 15000.**

4.2.1 Morphological Studies

Fig 4.2.1 (a, b, c) represents morphology of the nanofiber obatined under optimum spinnable composition of the composite by electrospinning technique. From the Figure it was found that the average diameter obtained for the composition 45:55 was found to be in the range of 250 -300 nm. Alignment of the nanofibers was found to be random with no bead formation thus making it a suitable feature for the bone tissue regeneration. It was also observed that there was no breakage of fibers in all the images obtained at different magnification.

4.2.2 Chemical Composition Analysis

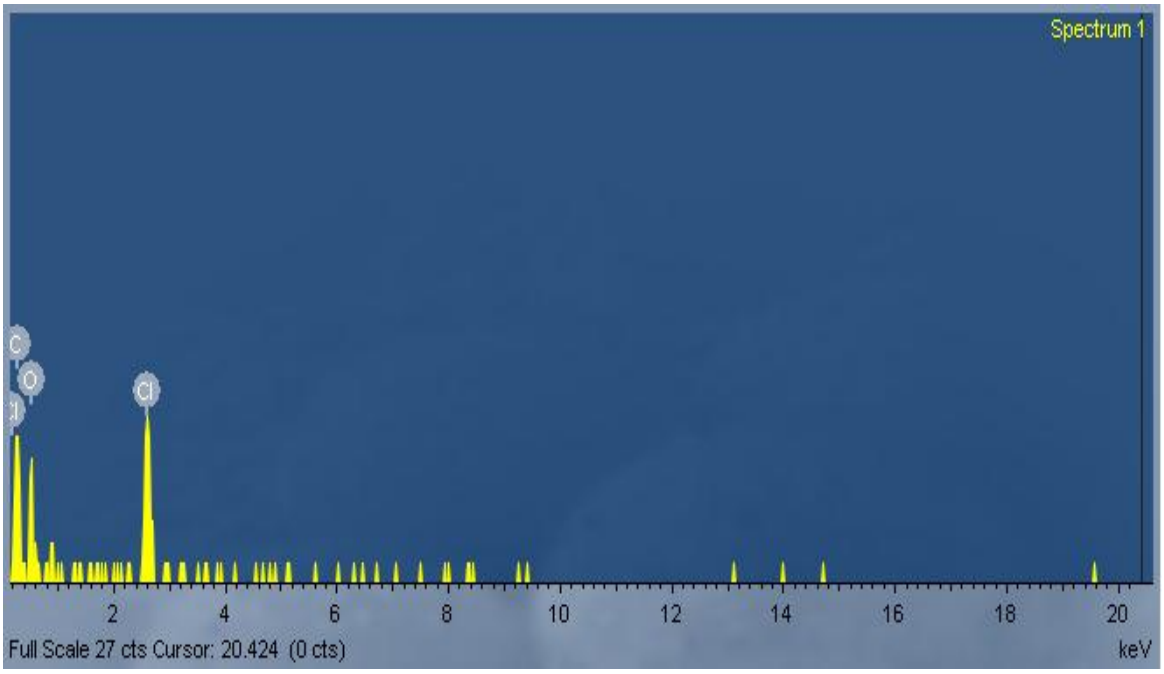


Fig 2 EDS analysis plot

Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	-31.18	0.1765	186.11	-315.51	140.38
O K	47.53	0.8472	-59.13	-218.56	-33.48
Cl K	22.00	0.8595	-26.98	-98.04	-6.89
Totals			100.00		

From the table below gives the chemical composition of the gelatin/PMMA composite as obtained from the EDS analysis. As the solvent used for preparing the solution was TCA and GAA larger peak showed the presence of higher amount of Chlorine. Similarly smaller peaks showed the presence of Carbon, oxygen and Hydrogen.

4.2.3Analysis of chemical Interaction

Fig 5. FTIR analysis peak

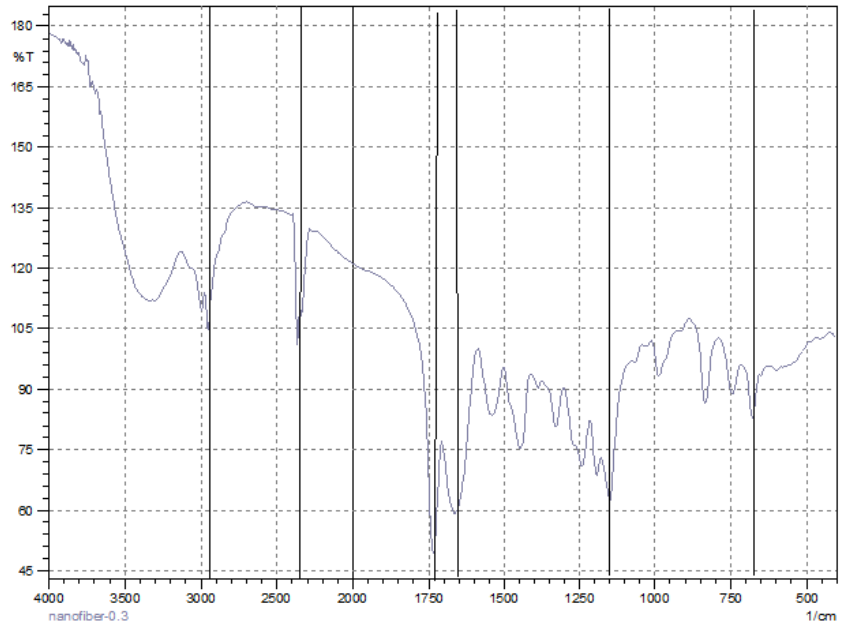


Fig 5 shows the FTIR spectra for the nanofibrous composites for composition gelatin: PMMA in 45:55 ratio.

For peaks obtained at different values are calculated from which can know the type of bond present. Peaks were obtained at 2500- 3000 which showed the presence of O-H bond. For 1750 – 1600 peak it showed the presence of N-H bond. Another major peak was obtained at 750- 1000 which confirmed the presence of C-H bond.

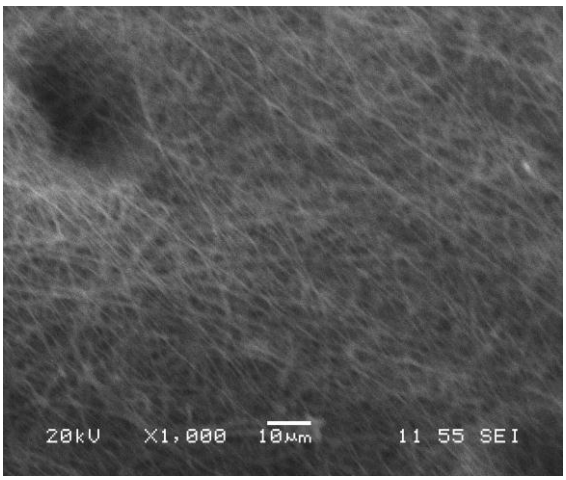
4.3 Effect of feed rate on the fiber morphology.

The effect of feed rate on the fiber morphology of gelatin:PMMA composite for 45:55 composition was also carried out. Three different feed rate was chosen for the study and interaction between different components and was analysed by FTIR and EDS technique.By varying the feed rate its effect on the fiber morphology was studied using scanning electron Microscopy.

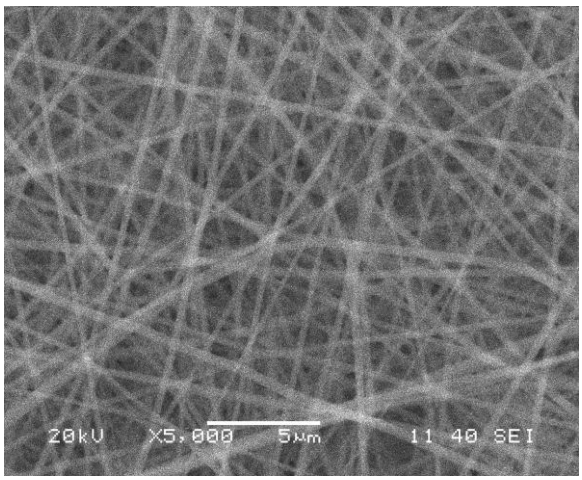
Sl.no.	**Gelatin:PMMA concn (% w/v)**	**Voltage applied (in kv)**	**Feed rate (Ml/h)**	**Fiber morphology**
1.	45:55	20-22	0.2	Fibres are non-aligned with no bead forr
2.	45:55	20-22	0.3	Fibers formed are nonaligned with no be formation with better surface morpholog
3.	45:55	20-22	0.4	Non-aligned fibers with bead formation.

4.3.1 Morphological Studies.

a) For feed rate 0.2 ml/hr



b) For feed rate 0.3 ml/hr



c) For feed rate 0.4 ml/hr

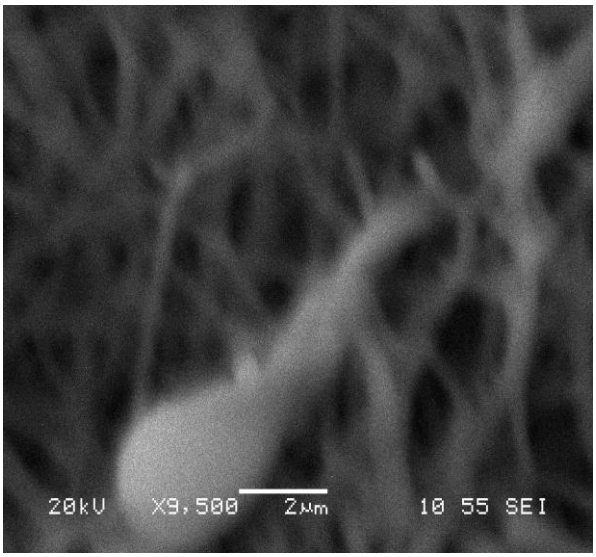


Fig 4.3.1 scanning electron micrograph obtained under different feed rate a) 0.2 ml/hr b) 0.3 ml/hr c) 0.4 ml/hr.

Fig 4.3.1 a, b, c shows different scanning electron microscopy images of the nanofibrous composites at varying feed rates of 0.3, 0.4 , 0.5 ml/hr. From the image Fig 4.3.1 it is observed that fiber obtained at feed rate 0.2 ml/hr had led to the formation of very thin and fine fiber . In fig 4.3.1.b fibers formed at 0.3 ml/hr showed morphology and diameter within the range to that of the collagen diameter without any bead formation. In case of Fig 4.3.1 c there were bead formation as the feed rate was increased. It was clearly viewed from the SEM diagrams that the alignment of fibers was random with higher bead formation and greater average diameter. Thus it was found that the average diameter did not change with the alteration in the gelatin composition and with the increasing in the feed rate there were bead formation which is not desired for the bone tissue regeneration.

4.3.2 Analysis of chemical composition.

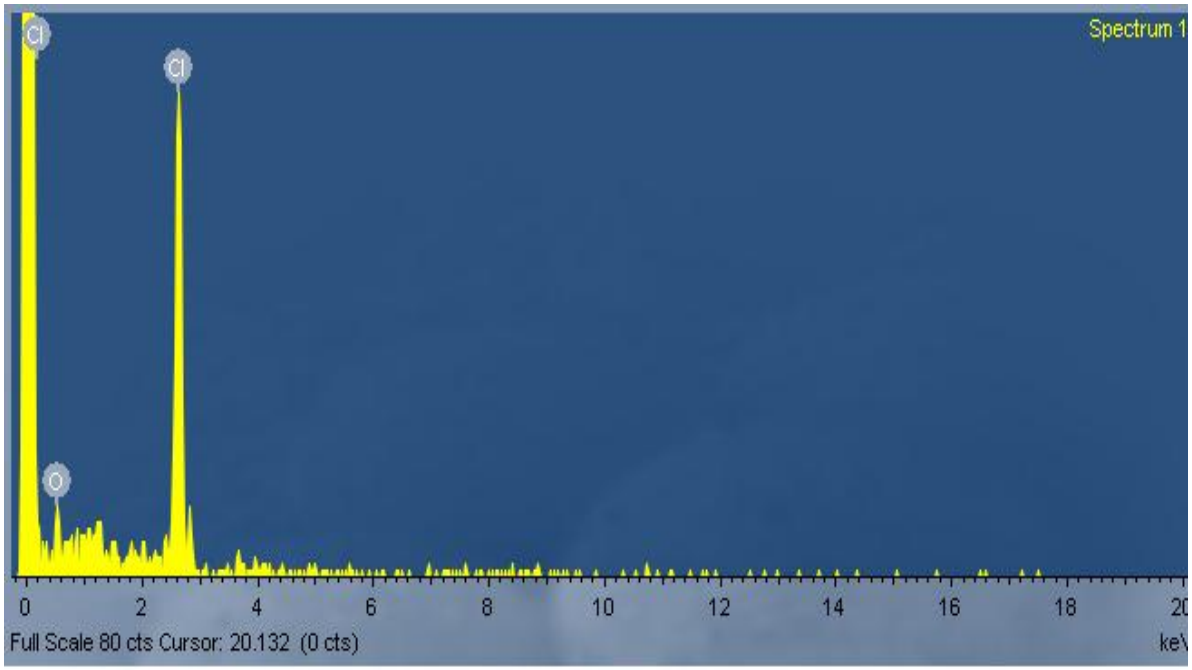
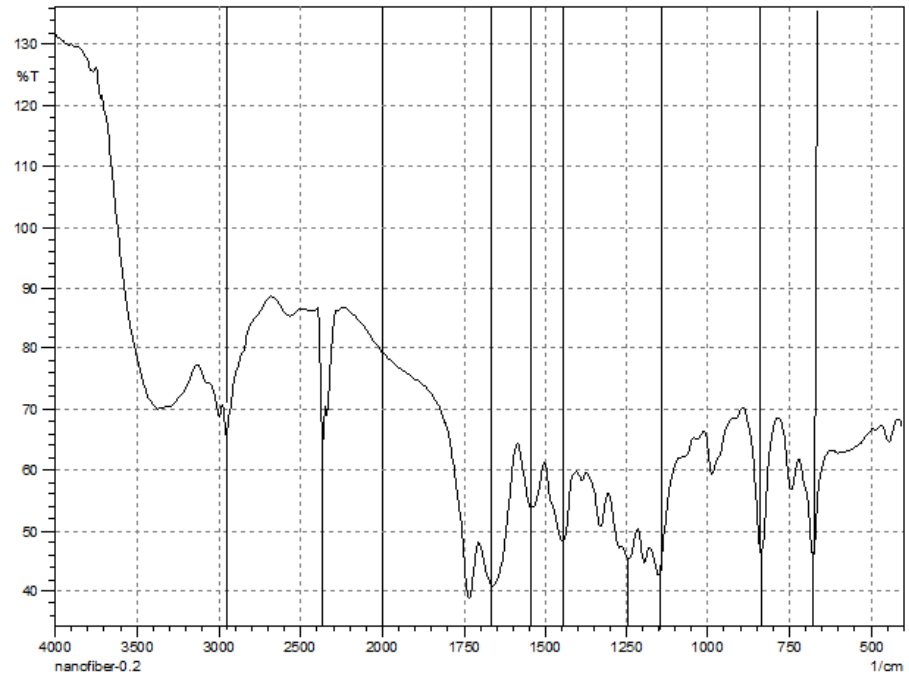


Fig 4.3.2 EDS Analysis

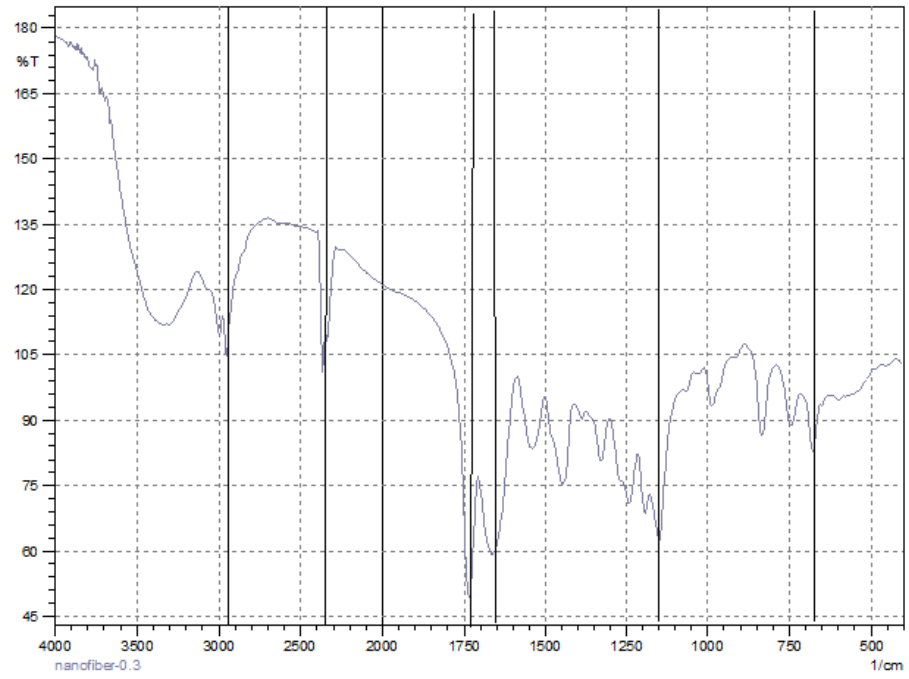
From EDS analysis , the chemical composition of the gelatin/PMMA composite was found. As the solvent used for preparing the solution was TCA and GAA larger peak showed the presence of higher amount of Chlorine. Similarly smaller peaks showed the presence of Carbon, oxygen and Hydrogen.

4.3.3 Analysis of chemical interaction.

a) FTIR spectra for nanofiber with feed rate 0.2ml/hr



b) FTIR spectra for nanofiber with feed rate 0.3 ml/hr.



c) FTIR analysis for nanofiber with feed rate 0.4ml/hr.

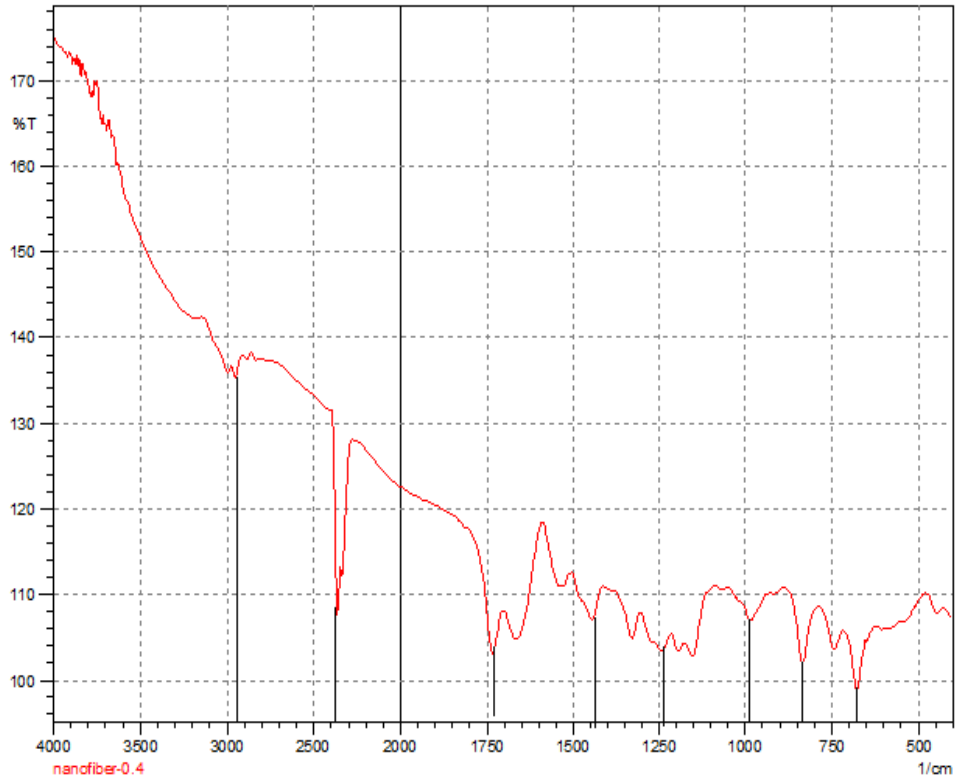


Fig 4.3.1 FTIR Analysis of nanofibrous composites formed under

a) Feed rate 0.2 ml/hr.

b) Feed rate 0.3 ml/hr.

c) Feed rate 0.4 ml/hr.

From FTIR analysis a plot was obtained for % transmittance vs wave number. For each peak obtained the value was calculated for the type of bond present can be known. Therefore in Fig. 4.2.3 peaks were obtained at 2500- 3000 which showed the presence of O-H bond. For 1750 – 1600 peaks it showed the presence of scissoring mode of N-H bond. Another major peak was obtained at 750- 1000 which confirmed the presence of C-H bond. Similarly in case of fig 4.2.3 a,b,c similar peaks were obtained at expected regions for different feed rates which confirmed the presence of C-H, C-O, N-H bonds. This FTIR analysis thus confirmed the interaction of PMMA and gelatin.

Chapter -5

CONCLUSION

In this study, composites with different ratios were prepared and analysed using SEM, FTIR, & EDS. This study was used to know the fiber alignment, morphology, chemical composition, chemical interaction. Furthermore previous experiments were carried out to find the optimum parameters to achieve nanofibers by electrospinning technique.

The conclusions are as follows –

1. Nanocomposites in the form of nanofibers were successfully prepared by electrospinning of gelatin with PMMA with varying ratios in solvent TCA and GAA.
2. It was first found that the fiber formed with electrospinning varied by varying processing parameters.
3. The best obtained nanofiber was found for the concentration 45:55 with the feed rate 0.3 ml/hr.
4. The diameters of the prepared nanofiber were between 50- 500 nm scale.
5. Effect of other processing parameters can be varied to get optimum results for the formation of nanofibrous composites.

Chapter 6

FUTURE WORK AND SUGGESTIONS.

1. Choosing one of the above compositions for the preparation of electrospun nanocomposites and characterizing for the mechanical properties.
2. Using another type of collector and solvent system for improving the fiber morphology.
3. Preparing nanofibers with other compositions with the aim of findind the best percentage of reinforcement.

Chapter 7

REFERENCES

1. Huang Zheng- Ming ,Zhang Y.Z., Ramakrishna S., Lim C.T., Electrospinning and mechanical characterization of gelatine nanofibers ,Polymer 45 (2004) 5361–53682.

2. Wanyao Xia, Wei Liu, Lei Cui, Yuanchun Liu, Wei Zhong, Deli Liu Juanjuan ,Wu Kienhui Chua, Yilin Cao, Electrospinning and mechanical characterization of gelatine nanofibers, 18 June 2004 in Wiley InterScience .

3. Engineered collagen-PEO nanofibers and fabrics. Huang L, Nagapudi K, Apkarian RP, Chaikof EL J Biomater Sci Polym Ed. 2001;12(9):979-93

4. Electrospinning of collagen nanofibers. Matthews JA, Wnek GE, Simpson DG, Bowlin GL Biomacromolecules. 2002 Mar-Apr; 3(2):232-8.

5. Runx2/Cbfa1-genetically engineered skeletal myoblasts mineralize collagen scaffolds in vitro. Gersbach CA, Byers BA, Pavlath GK, Guldberg RE, García AJ. Biotechnol Bioeng. 2004 Nov 5;88(3):369-78

6. Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. Zhang Y, Ouyang H, Lim CT, Ramakrishna S, Huang ZM. J Biomed Mater Res B Appl Biomater. 2005 Jan 15;72(1):156-65.

7. Electrospinning Bombyx mori silk with poly(ethylene oxide). Jin HJ, Fridrikh SV, Rutledge GC, Kaplan DL. Biomacromolecules. 2002 Nov-Dec;3(6):1233-9

8. Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. Yang F, Murugan R, Wang S, Ramakrishna S. Biomaterials. 2005 May;26(15):2603-10

9. Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer. Verreck G, Chun I, Rosenblatt J, Peeters J, Dijck AV, Mensch J, Noppe M, Brewster ME. J Control Release. 2003 Oct 30;92(3):349-60

10. Biological response of chondrocytes cultured in three-dimensional nanofibrous poly(epsilon-caprolactone) scaffolds. Li WJ, Danielson KG, Alexander PG, Tuan RS. J Biomed Mater Res A. 2003 Dec 15;67(4):1105-14.

11. Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend. Kenawy el-R, Bowlin GL, Mansfield K, Layman J, Simpson DG, Sanders EH, Wnek GE.

12. Yannas IV. Natural materials. In: Ratner BD, Hoffman AS, Schoen FJ, et al., editors. Biomaterial science: an introduction to materials in medicine 2. San Diego: Elsevier Academic Pr; 2004. pp. 127–136.

13. Zhang Y, Ouyang H, Lim CT, Ramakrishna S, Huang ZM ; Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. J Biomed Mater Res B Appl Biomater. 2005 Jan 15;72(1):156-65.

14. Peter SJ, Miller MJ, Yasko AW, et al. Polymer concepts in tissue engineering. J Biomed Mater Res (Appl Biomater) 1998;43:422–7.

15. Cooper SL, Visser SA, Hergenrother RW, et al. Polymer. In: Ratner BD, Hoffman AS, Schoen FJ, et al., editors. Biomaterial science: an introduction to materials in medicine. 2. San Diego: Elsevier Academic Pr; 2004. pp. 67–79.

16. Ma Z, Kotaki M, Yong T, He W, Ramakrishna S : Surface engineering of electrospun polyethylene terephthalate (PET) nanofibers towards development of a new material for blood vessel engineering. Biomaterials. 2005 May;26(15):2527-36.

17. Mo X, Weber HJ. Electrospinning P(LLA-CL) nanofiber: a tubular scaffold fabrication with circumferential alignment. Macromol Symp. 2004;217:413–16.

18. Katti DS, Robinson KW, Ko FK, et al. Bioresorbable nanofiber based systems for wound healing and drug delivery: optimization of fabrication parameters. J Biomed Mater Res. 2004;70B:286–96.

19. Uematsu K, Hattori K, Ishimoto Y, Yamauchi J, Habata T, Takakura Y, Ohgushi H, Fukuchi T, Sato M: Cartilage regeneration using mesenchymal stem cells and a three-dimensional poly-lactic-glycolic acid (PLGA) scaffold. Biomaterials. 2005 Jul;26(20):4273-9

20. Bruder SP, Caplan AI. Bone regeneration through cellular engineering. In: Lanza RP, Langer R, Vacanti J, editors. Principles of tissue engineering. San Diego: Academic Pr; 2000. pp. 683–96.

21. Bruder SP, Caplan AI. Bone regeneration through cellular engineering. In: Lanza RP, Langer R, Vacanti J, editors. Principles of tissue engineering. 2. San Diego: Academic Pr; 2000. pp. 683–96.

22. Uematsu K, Hattori K, Ishimoto Y, Yamauchi J, Habata T, Takakura Y, Ohgushi H, Fukuchi T, Sato M: Cartilage regeneration using mesenchymal stem cells and a three-dimensional poly-lactic-glycolic acid (PLGA) scaffold. Biomaterials. 2005 Jul;26(20):4273-9.

23. Amay HRR, Zhang M. Preparation of porous hydroxyapatite scaffolds by combination of the gel casting and polymer sponge methods. Biomaterials . 2003; 24:3292–302.

24. Bhattarai N, Edmondson D, Veiseh O, et al. Electrospun chitosan-based nanofibers and their cellular compatibility. Biomaterials. 2005 In press.

25. Price RL, Waid MC, Haberstroh KM, Webster TJ. Selective bone cell adhesion on formulations containing carbon nanofibers. Biomaterials. 2003 May;24(11):1877-87.

26. Lia Mengyan, Guob Yi, Weib Yen, MacDiarmidc Alan G, Lelkes Peter I. Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. Biomaterials 27 (2006) 2705–2715.

27. Hae-Won Kim, Hye-Sun Yu, Hae-Hyoung Lee. Nanofibrous matrices of poly(lactic acid) and gelatin polymeric blends for the improvement of cellular responses. Department of Biomaterials Science, School of Dentistry, Dankook University, Cheonan 330-714, Korea

28. Wan Meng , Zhi-Cai Xing , Kyung-Hye Jung , Se-Yong Kim , Jiang Yuan , Inn-Kyu Kang , Sung Chul Yoon, Hong In Shin. Synthesis of gelatin-containing PHBV nanofiber mats for biomedical application. J Mater Sci: Mater Med (2008) 19:2799–2807.